PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 47/48

A2

(11) International Publication Number: WO 98/20905

(43) International Publication Date: 22 May 1998 (22.05.98)

(21) International Application Number: PCT/GB97/03064

(22) International Filing Date: 7 November 1997 (07.11.97)

(30) Priority Data:
08/745,335 8 November 1996 (08.11.96) US

(71) Applicant *(for all designated States except US)*: BIOMEDICAL FRONTIERS, INC. [US/US]; 1095 Tenth Avenue, S.E., Minneapolis, MN 55414 (US).

(71) Applicant *(for KG only)*: SEABORN, George, Stephen [GB/GB]; Edward Evans & Co., Chancery House, 53–64 Chancery Lane, London WC2A 1SD (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: DRAGSTEN, Paul, R. [US/US]; 30 Choctaw Circle, Chanhassen, MN 55317 (US). HALLAWAY, Philip, E. [US/US]; 1458 West 35th Street, Minneapolis, MN 55408 (US). HEDLUND, Bo, E. [US/US]; 2049 16th Street, N.W., New Brighton, MN 55112 (US).

(74) Agent: EDWARD EVANS & CO.; Chancery House, 53–64 Chancery Lane, London WC2A 1SD (GB).

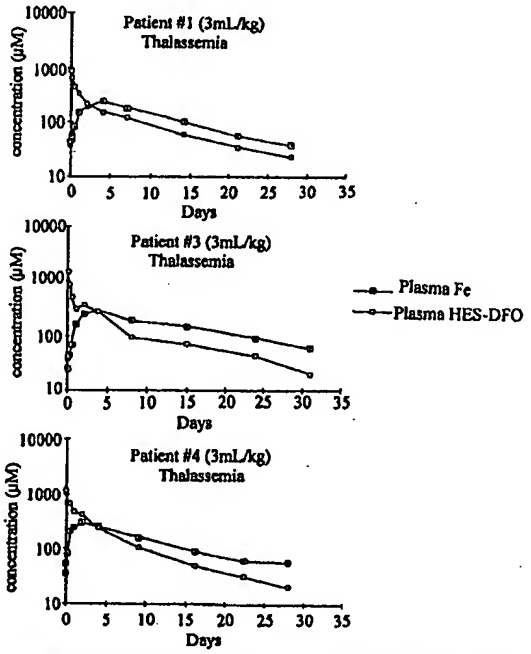
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: TREATMENT OF IRON OVERLOAD DISORDERS

(57) Abstract

The invention relates to methods of treating iron overload disorders by administering a conjugate of a chelator covalently bonded to a water soluble carrier.



Plasma iron and HES-DFO concentrations following dosing in the three patients who received 3 mL/kg.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

TREATMENT OF IRON OVERLOAD DISORDERS

Background of the Invention

Iron overload disorders result from toxicity that occurs when the level of iron in a particular tissue exceeds the ability of this tissue to safely store this highly reactive, essential metal. Iron is normally stored intracellularly in the form of ferritin, a protein whose synthesis is induced upon influx of iron. When the storage capacity of ferritin is exceeded, pathological quantities of metabolically active iron are released intracellularly in the form of hemosiderin (partially degraded ferritin) and free iron within an expanded labile pool. This metabolically active iron catalyzes the formation of free radicals, which damage membrane lipids and other macromolecules, leading to cell death and eventually organ failure. The heart is more susceptible to iron toxicity than the liver because the liver produces much more ferritin.

A variety of iron overload disorders have been characterized. Iron overload disorders include thalassemia, sickle cell disease, Diamond-Blackfan anemia, hereditary (idiopathic) hemochromatosis, hereditary transferrin deficiency, thalassemia syndromes, hereditary hypochromic anemia, African hemosiderosis, Kaschin-Beck disease, transfusional hemosiderosis, alcoholic cirrhosis with hemosiderosis, porphyria cutanea tarda, acquired hemolytic anemia, ineffective erythropoiesis, or pyridoxine-responsive

anemia. In hereditary hemochromatosis, increased amounts of iron are absorbed from the diet and deposited mainly in hepatocytes and in parenchymal cells of other organs. Increased iron deposition in reticuloendothelial cells usually does not occur in this disease until iron overload is far advanced. In iron overload disorders treated by transfusion, overload usually is characterized first by excessive accumulation of hemosiderin in the reticuloendothelial system. In such cases, involvement of parenchymal cells in the liver and other organs leading to tissue damage typically occurs in more advanced cases.

Thalassemia presents an example of the effects of an iron overload disorder and the difficulty in treating these disorders. Thalassemia patients accumulate iron through chronic transfusion therapy. Since humans lack an effective physiological mechanism for excretion of iron, these patients accumulate an increasing load of iron unevenly through the body in various tissues and organs. Since each unit of red cells contains 200 to 250 mg of elemental iron, patients on chronic transfusion programs will accumulate significant amounts. By age 12, a thalassemia patient receiving appropriate transfusion therapy will have stored 55 or more grams of excess iron in various tissues that would have a normal overall load of approximately 2 grams. In addition, thalassemia patients, who suffer from ineffective erythropoiesis, absorb iron from the gastrointestinal

tract at an increased rate, further adding to their iron burden.

In the absence of a physiological means for excreting this excess iron, chelation therapy has become an essential facet of good clinical management in this disease. Deferoxamine (DFO, desferrioxamine, deferrioxamine, Desferal®) has been the chelator of choice for therapy of thalassemia and other iron overload disorders. More than 20 years of use of deferoxamine has highlighted the benefits and drawbacks of therapy with this chelator.

Deferoxamine is a naturally occurring compound produced by *Streptomyces pilosus* with an affinity for iron comparable to that of transferrin, the specific iron-binding protein of blood. Because deferoxamine is poorly absorbed from the gastrointestinal tract, it must be administered parenterally. Deferoxamine has short vascular half-life, only about 5-10 min. after intravenous infusion, so long duration infusion is required to decrease iron levels. As a result of the short half-life, deferoxamine therapy involves continuous infusion or frequent intramuscular injections, which may cause pain and/or induration at the injection site. It is believed that deferoxamine *in vitro* enters certain cells such as hepatocytes where it binds iron to form a complex. The complex of iron with deferoxamine exits cells and is excreted both in the urine and feces.

Treatment with deferoxamine has been shown to produce net negative iron balance, to reduce tissue iron stores, to delay or prevent iron-induced organ damage and to improve the survival of patients with transfusional iron overload. Furthermore, patients compliant with chelation therapy are less likely to develop cardiac complications than patients who are not compliant or who start therapy at an older age. These findings suggest that iron chelation therapy with deferoxamine, begun or intensified before the time of irreversible tissue damage, can improve organ function.

Perhaps the most important aspect of ensuring adequate chelation is the fostering of patient compliance. Compliance is generally good during infancy and childhood when parents are responsible for drug administration, but falls off in many patients during adolescence. Reasons for decreased compliance include poor acceptance of the fact that their illness is chronic; a sense of invulnerability; embarrassment and discomfort associated with using an infusion pump; the complexity of the chelation regimen; and lack of immediate symptomatic relief. At the same time, most patients now recognize the fact that poor compliance correlates with organ failure and ultimately death.

Despite the benefits of deferoxamine therapy in thalassemia patients, there are still significant concerns regarding its safety, particularly at high does. Regimens in which the dose exceeds 50 mg/kg

administered intravenously over 12 hours, or standard subcutaneous dose regimens in well chelated younger patients with minimal iron burdens have been associated with a variety of toxicities (e.g., neurological, pulmonary, renal, bone, visual, auditory and growth abnormalities).

These observed toxicities lead to a variety of concerns over therapy with deferoxamine. For example, it is uncertain at what age children should be exposed to the burdensome protocol of daily, 8-12 hour, subcutaneous administration of this toxic compound. In many cases it is unclear how to adjust doses to maintain safety and to provide adequate decreases in iron levels. Furthermore, it is often difficult to convince patients to comply with the burdensome, and often toxic, treatment protocol that may show little immediate benefit in the patient's life. In extreme cases, patients may require weeks to months of continuous treatment as well as placement of central venous lines to permit continuous or intermittent high-dose intravenous therapy on an ambulatory basis, either alone or as a supplement to subcutaneous administration.

Small molecular weight deferoxamine escapes from a patient's vascular system where it can pick up iron from a variety of locations for later excretion. In most iron overload disorders, it is believed that the harmful iron resides inside cells and outside of the vascular system. Therefore the common belief is that a chelator that is retained in the circulation

and excluded from cells could not effectively treat an iron overload disorder. Furthermore, the majority of iron in plasma is in the form of a complex with transferrin or ferritin. Deferoxamine alone does not remove significant amounts of iron from either transferrin or ferritin. Hence, it is believed that deferoxamine, if confined to the circulation, could not access and complex the cellular and tissue iron stores common to iron overload disorders, and would not contribute to iron excretion. So it is believed that treatment with a chelator that is confined to the vasculature would be ineffective for treating iron overload disorders because it could not get to what are believed to be harmful iron stores and because it would not remove iron bound to transferrin or ferritin from the circulation.

Given the shortcomings of current treatments described above, a need exists for a method to treat iron overload disorders that overcomes the problems with toxicity, burdensome treatment protocol, compliance, and like problems with current treatment regimes using chelators. Such a method would include administering a medical composition that is resistant to *in vitro* or *in vivo* degradation, that has a prolonged half-life in the patient, and that is excreted when iron-loaded.

Summary of the Invention

The present invention relates to treatment of an iron overload disorder in a patient and

meets the needs described above. The treatment includes administering to the patient a covalently bonded conjugate of a chelator and a water soluble carrier. Hereinafter a conjugate of a chelator and a water soluble carrier is referred to as a chelator conjugate or a conjugate. The invention is effective to treat iron overload disorders in patients such as mammals, preferably humans, including a child, an adolescent or an adult.

The therapeutic agent of the invention, a conjugate formed from a chelator and a water soluble carrier, can be administered by means compatible with administration of such agents to patients. Preferably, administration is parenteral. More preferably, administration is intravenous.

The water soluble carrier of the invention is a large molecule that has characteristics effective to improve the treatment with the chelator by, for example, increasing efficacy, prolonging the circulating half-life, decreasing toxicity, and the like. Preferably, the water soluble carrier is starch, a starch derivative, dextran, or hyaluronic acid. In a preferred embodiment, the water soluble carrier is hydroxyethyl starch.

The chelator of the invention is effective to chelate iron so that the iron can be excreted from the body of the patient. In a preferred embodiment, the chelator is deferoxamine.

In a preferred embodiment, a conjugate of deferoxamine with hydroxyethyl starch (HES-DFO) is

administered to decrease iron load in a patient suffering from an iron overload disorder. Preferably, HES-DFO is administered as a solution with chelator conjugate concentration at about 100 g/L to about 200 g/L. Preferably, HES-DFO chelator conjugate is administered as a solution with a total deferoxamine concentration of about 26 mM to about 50 mM, preferably to about 30 mM. Preferably, HES-DFO chelator conjugate is administered at about 3 mL/kg to about 9 mL/kg. Preferably, administration of the HES-DFO chelator conjugate achieves plasma levels of chelator of about 0.5 mM to about 3 mM.

A conjugate of deferoxamine can be administered by a route that delivers effective quantities of chelator conjugate to reduce iron levels in the body of the patient. Preferably, administration is by intravenous infusion for a period of less than about 3 hours. More preferably, the intravenous infusion is for a period of about 1 hour.

A chelator conjugate of the invention can be administered as a solution, suspension, or emulsion, or similar liquid based form. Preferably, the chelator conjugate is administered in a pharmaceutically accepted vehicle. Preferably, the pharmaceutically acceptable vehicle is water including about 0.9% sodium chloride. More preferably, the chelator conjugate is administered as a solution with chelator conjugate concentration of about 5 g/L to about 250 g/L. More preferably, the chelator conjugate is administered as a solution with a total

chelator concentration of about 5 mM to about 100 mM. More preferably, plasma levels of chelator are about 0.1 mM to about 15 mM. More preferably, the chelator conjugate is administered at about 1 mL/kg to about 10 mL/kg.

The method of treating iron overload disorders can include other steps effective to maintain the health of the patient or to monitor the efficacy of the treatment.

Brief Description of the Drawings

Figure 1 shows graphs of plasma iron and HES-DFO concentrations in thalassemia patients 1, 3 and 4 following dosing with 3 mL/kg of HES-DFO.
Figure 2 shows graphs of plasma iron and HES-DFO concentrations in four patients who received 5 mL/kg of HES-DFO.
Figure 3 shows a graph of cumulative urinary iron excretion at the 3 mL/kg dose in patients 1, 3 and 4.
Figure 4 shows a graph of cumulative urinary iron excretion at 5 mL/kg dose of HES-DFO in patients 5-8.
Figure 5 shows a graph of cumulative urinary HES-DFO excretion at 3 mL/kg dose in patients 1, 3 and 4.
Figure 6 shows a graph of cumulative urinary HES-DFO excretion at the 5 mL/kg dose in patients 5-8.

Detailed Description of the Invention

Chelators for Therapy of Iron Overload Disorders

Iron overload disorders can be treated by administration of a chelator. Chelation therapy has in the past focused on small molecule iron chelating agents, such as deferoxamine. In the present

invention, chelator used for treatment of an iron overload disorder is covalently bonded to water soluble carrier to form a chelator conjugate.

Iron chelators that have been studied in the treatment of iron overload disorders include deferoxamine (deferrioxamine or desferrioxamine), 2,3-dihydroxybenzoic acid, DTPA, rhodotorulic acid, cholylhydroxamic acid, ethylene diamine-N,N^1-bis(2-hydroxyphenylacetic acid), isoniazid-pyridoxal hydrozone, 1,2-dimethyl-3-hydroxypyrid-4-one and nitrilotriacetate. These chelators can be used alone or in combination.

The Water Soluble Carrier

Covalently binding a chelator to a water soluble carrier has been discovered to be advantageous for several reasons. For example, binding to the water soluble carrier can alter the distribution of the chelator in the patient. Although not limiting to the present invention, it is believed that the conjugate of chelator and water soluble carrier is retained in the circulation to a greater degree than the chelator alone. Additional advantageous features of a chelator conjugate can include diminished toxicity, increased stability of the chelator in solution, formulations and plasma, and greater efficiency of iron chelation in vivo (i.e., more moles of iron bound per mole of chelator administered). It will be understood from the below reported experimental results that behavior of the bound

chelating conjugate is not always readily predictable from data concerning the non-bound or non-immobilized chelator.

The water soluble carrier is preferably a natural polymer, a modified natural polymer, or another pharmaceutically acceptable organic polymer. Such water soluble carriers include polysaccharides such as dextrans and hyaluronic acid, starch and starch derivatives, and proteins such as human serum albumin, and the like. Polymer starting materials such as the dextrans, human albumin and plasma protein fraction are commercially available as water-soluble preparations or as solutions. See Remington's Pharmaceutical Sciences, A. Osol., ed., Mack Publishing (16th ed. 1980) at pages 759-761. Water soluble carriers of the invention include those described in U.S. Patent Nos. 4,863,964, 5,217,998, and 5,268,165, the disclsoures of which are incorporated herein by reference.

The water soluble carrier is sufficiently stable to carry the chelator in the patient for a sufficient time that it is effective to treat the iron overload disorder. In addition, the water soluble carrier is sufficiently well-tolerated and non-toxic that the patient has no unacceptable adverse reactions to the treatment. Preferably, the conjugate of water soluble carrier with the chelator has fewer side effects and lower toxicity than the chelator alone.

Preparing the Chelator Conjugate

There are several ways in which a chelator can be covalently bonded to the water soluble carrier to form a chelator conjugate. The chelator is bound to the water soluble carrier in a manner such that its chelating ability, as measured *in vitro*, remains substantial, and preferably on the order of the non-conjugated chelator. One preferred way to form conjugates of chelators with a water soluble carrier is to bind amino groups, such as a terminal amino group of deferoxamine, to the water soluble carrier. Such an amino group can form a covalent bond with a carboxyl group on a water soluble carrier to form an amide linkage.

Preferably, an amino group of the chelator will form a covalent bond with an aldehyde moiety. In an initial reaction, the amine on the chelator reacts with the aldehyde to form a Schiff base, and the Schiff base is reduced in a second reaction to yield more stable covalent linkage. Aldehydic groups can be introduced into the polymer substrates by known techniques, e.g. by the oxidation of carbohydrates or other diols to dialdehydes with sodium metaperiodate. See, for example, M.B. Wilson, et al. in Immunofluorescence and Related Staining Techniques, W. Knapp et al., eds., Elsevier/North Holland Biomedical Press (1978) at page 215', Flemming et al., Acta Biol. Med. Ger., 30, 177 (1973); and, S.-C. Tam et al., in P.N.A.S. USA, 73, 2128 (1976). In some applications, the terminal amino group on deferoxamine can also be

bonded to an amino group on the polymer directly, by the use of a dialdehyde linking agent such as glutaraldehyde, followed by reduction, e.g., with sodium borohydride.

More preferred chelator conjugates are prepared by covalently bonding deferoxamine to a pharmaceutically-acceptable organic polymer. Methods for the preparation of deferoxamine (N-[5-[3[(5-aminopentyl) hydroxycarbamoyl] propionamido]pentyl]-3-[[5-(N-hydroxyacetamido) pentyl] carbamoyl]propionohydroxamic acid) and its pharmaceutically-acceptable salts have been disclosed, e.g., by Prelog et al., in Helv. Chim. Acta., 45, 631 (1962); Bickel et al., Helv. Chim. Acta. 46 1385 (1963); in German Pat. Spec. 1,186,076 and in United States Patent Nos. 4,419,365, 4,987,253, and 5,493,053, the disclosures of which are incorporated by reference herein. Such salts include the acid addition salts of methane sulfonic acid, phosphoric acid, acetic acid, lactic acid, tartaric acid, citric acid, and the like.

Methods for preparing chelator conjugates of the invention include the methods described in U.S. Patent Nos. 4,863,964, 5,217,998, and 5,268,165, the disclosures of which are incorporated herein by reference.

The mole ratios of deferoxamine/water soluble carrier attainable by reactions with carboxyl or carbonyl groups can vary widely, depending on factors such as the number of reactive groups on the

polymer, steric hindrance, rate and extent of Schiff base or amide formation, and the like. More than one molecule of chelator can be attached to each molecule of water soluble carrier. As an example, about 0.6-0.7 g of deferoxamine can be bonded to about 2.5 g of reacted Dextran 40, via reaction of the deferoxamine with aldehyde groups introduced into the dextran, followed by reduction. When the water soluble carrier is hydroxyethyl starch, about 0.2g to about 0.4g of deferoxamine can be bonded to about 1g of hydroxyethyl starch.

Preferably, when the chelator is deferoxamine, the water soluble carrier is hydroxyethyl starch. More preferably, the hydroxyethyl starch has an average molecular weight of between about 50 kD and about 200 kD as measured by gel permeation chromatography using Pullulan molecular weight standards. The corresponding average molecular weights determined using light scattering methods are about 100 kD to about 150 kD.

The preferred preparations of chelator conjugates for use *in vivo* provide preferred characteristics for pharmaceutical use. The chelator conjugate preferably is sufficiently soluble for ease of introduction. The chelating moiety of the chelator conjugate is effective as a chelator, even *in vivo*. The preferred chelator conjugate shows improved vascular retention and is efficacious in generating iron excretion from animals. The preferred chelator conjugate is not substantially toxic, at least at or

near therapeutic levels, and preferably not within about 5 to 10 times therapeutic levels. The preferred water soluble carrier does not cause significant side reactions, and thus is selected from polymers which are biocompatible.

Administering the Chelator conjugate

The chelator conjugate can be delivered by a variety of routes effective to gain circulating and local levels sufficient to reduce detrimental stores of iron and to increase excretion of iron. Typical routes of administration would be parenteral, such as intravenous or subcutaneous. The chelator conjugate is preferably administered as a solution or suspension in an aqueous solvent that is compatible with administration to patients such as animals, mammals or humans. Preferably chelator conjugates are administered, as solutions, parenterally, such as by intramuscular, subcutaneous, or intravenous injection or infusion, or via buccal, pulmonary, rectal or vaginal routes. The appropriate dose will be adjusted in accord with appropriate clinical factors in hte treating physician's judgment including: the iron overload disorder to be treated; the patient or patient's age, size and weight; the mode of administration; binding capacity of the chelator, and the like. For example, see B. Modell et al., in The Clinical Approach to Thalassemia, Grone and Stratton, New York (1984) at chapter 13. General methods for administration of chelator conjugate include those

described in U.S. Patent Nos. 4,863,964, 5,217,998, and 5,268,165, the disclosures of which are incorporated herein by reference.

Preferably, the chelator conjugate is administered as a solution with a chelator conjugate concentration of about 5 g/L to about 250 g/L. Such a solution will include a total chelator concentration of about 5 mM to about 100 mM. Typically, when the solution of the chelator conjugate is administered intravenously, it is administered at a volume of about 1 mL/kg to about 30 mL/kg. Such administration is effective to achieve plasma levels of chelator between 0.1mM and about 15 mM.

Steps in the Method

The method for treating an iron overload disorder includes the step of administering the chelator conjugate to the patient, but it can include additional steps as well. Additional steps may be desirable to evaluate the course or efficacy of treatment, to monitor iron levels, to detect potential adverse effects, to monitor clinical chemistry or vital signs, and the like. In some instances, the practitioner overseeing the treatment could monitor the effectiveness of the treatment by additional steps such as monitoring plasma iron concentration, determining levels of plasma non transferrin bound iron, monitoring total body iron burden, monitoring urinary iron excretion or concentration, measuring iron content of a tissue such as liver, or making

laboratory and clinical assessments of the patient. Such additional steps can be added at the discretion of the practitioner to evaluate patient progress or compliance with therapy, and for other like purposes.

Treating Iron Overload Disorders with Chelator Conjugate

The approach to administration of a chelator conjugate for treatment of an iron overload disorder will depend on the characteristics of the iron overload disorder. Chelator conjugate can be effective to treat iron overload disorders with various origins. For example, many iron overload disorders are hereditary, but they arise from other causes as well. Chelator conjugate can be effective against various effects on iron storage presented by different iron overload disorders. For example, an iron overload disorder typically includes higher than normal circulating levels of iron, but they also result in various defects in iron storage and metabolism within an animal. These defects in iron storage and metabolism result in detrimental effects of the disorder, which typically arise from the amount and disposition of iron in tissues.

A chelator conjugate can prevent these detrimental effects and reduce undesirable disposition of iron by increasing excretion of iron from stores throughout the body, such as in the liver. Excess iron is often stored in the liver, which can result in enlargement of the liver and, in more severe instances, cirrhosis. Eventually, the cirrhosis

progresses to hepatic failure. In iron overload disorders there can be as much as 5 to 50 excess grams of iron stored in the body, and much of it in the liver. Excess iron can also accumulate in and detrimentally affect the skin, pancreas, joints, skeletal system, heart, gut, immune system, nervous system, and hematopoietic system. Treatment with a chelator conjugate can reduce tissue levels of iron.

Administering a chelator conjugate can increase iron excretion in patients with thalassemia. In thalassemia, it is believed that iron collects in a pool derived from catabolism of non-viable erythrocytes produced as a result of the disease, and from normal turnover of transfused erythrocytes. The catabolism is believed to occur in reticuloendothelial cells. These cells can collect in the spleen and, probably, in the marrow as well. Thalassemia also results in a large pool of iron in the liver, which is harmful. Conventional chelator therapy for thalassemia, as is typical of iron overload disorders, is believed to depend on the chelator leaving the circulation and entering the iron-laden tissue, such as the liver and reticuloendothelial cells, to chelate the iron. The chelator-iron complex is then excreted. Deferoxamine, for example, is known to enter hepatocytes, chelate iron, and then be excreted. It is conventionally believed that treatment of iron overload disorders requires a chelator that can enter tissue to gain access to the iron to allow chelation of the iron.

Hence, prior to the invention, it was not expected that a conjugate of a chelator and a water soluble carrier would be effective to treat iron overload disorders. Linking a chelator to the water soluble carrier forms a chelator conjugate that is largely retained in the circulation, the chelator conjugate does not enter the tissues in significant amounts. Since the chelator conjugate could not enter the iron-laden tissue, the chelator moiety on the chelator conjugate would not have access to iron in the tissue, and it was expected that the chelator conjugate would not decrease iron levels. Surprisingly, it has been discovered that administration of a chelator conjugate results in decreased iron levels and/or increased iron excretion in patients with iron overload disorders. Although not limiting to the present invention, it is believed that the efficacy of chelator conjugate administration may be related to the prolonged half-life of the conjugate in the circulation compared to unconjugated chelator.

Chelator conjugate is effective to treat a variety of iron overload disorders such as Diamond-Blackfan anemia, sickle cell disease, and thalassemia. In Diamond-Blackfan syndrome, it is believed that iron collects in tissues such as liver, heart, spleen and bone marrow. Diamond-Blackfan patients typically receive transfusions every two to four weeks. Each transfusion introduces additional iron in transfused erythrocytes. In the absence of a chelator, the iron

taken in through the transfusions is not excreted in significant amounts. Surprisingly, although the iron stores in Diamond-Blackfan anemia are in the liver, heart, spleen, and bone marrow, a chelator conjugate that is largely retained in the circulation is effective to increase iron excretion in patients with this disorder.

Patients with sickle cell anemia can require repeated transfusions to reduce the likelihood of a sickling episode. Sickle cell anemia results in iron overload due to metabolism of injected erythrocytes and defective "sickle" erythrocytes. Excess iron deposition occurs in the liver, spleen, bone marrow, kidney, heart, and other organs. Once again, based on the conventional beliefs about chelator therapy, it is unexpected that a chelator that is confined largely to the circulation would be effective in treating iron overload due to transfusion treatment of sickle cell disease. However, treatment with chelator conjugate is effective to treat iron overload disorders including those with deposited excess iron in the tissues such as thalassemia, Diamond-Blackfan anemia, and sickle cell disease.

Benefits of Administering a Chelator conjugate

One benefit of administering a chelator conjugate to treat an iron overload disorder can be reducing the total body iron burden in the patient. However, monitoring total body iron burden is difficult and can require invasive tissue biopsy of

the target organ or expensive apparatus such as a superconducting quantum interference device (SQUID). In addition, the methods of monitoring require up to about 6 months or one year to show significant differences. An alternative way to determine the benefit of administering a chelator conjugate is to demonstrate negative iron balance in the patient. The amount of iron introduced into patients through transfusion therapy can be routinely determined. Monitoring iron excretion in the feces, urine, or both can show whether the amount excreted is matching or exceeding the amount of iron introduced by transfusion. To gain net negative iron balance, the amount excreted must exceed the amount taken in.

Administering chelator conjugate can result in reduced total body iron burden and increased excretion of iron. Iron bound to the chelator conjugate can be excreted either in the feces or in the urine. Treatment with a chelator conjugate of a chelator and a water soluble carrier can increase excretion by either or both routes. For example, administration of a chelator conjugate of the invention can increase urinary excretion by about 20 mg to about 100 mg of urinary iron per 7 day period. In addition, chelator conjugate treatment can reduce levels of plasma non-transferrin-bound iron. Levels of plasma non-transferrin-bound iron can be eliminated or reduced to levels near zero for a substantial period after administration of the chelator conjugate. For example, plasma non-transferrin-bound iron can be

reduced to about zero for a period of, or more than, about 1 hour up to about 4 days. In particular, administration of chelator conjugate can have several effects that result in reduced iron load in a patient suffering from thalassemia. For example, treatment can result in reduced plasma non-transferrin-bound iron and increased urinary iron excretion.

These beneficial effects of administration of a chelator conjugate on increasing iron excretion and decreasing body levels of iron occur without unacceptable adverse changes in other measures of the patient's health. For example, administration of the chelator conjugate causes no unacceptable adverse changes in clinical chemistry, urinalysis, hematology, vital signs, or combinations of these measures. Clinical chemistry measurements such as levels of albumin, alkaline phosphatase, bicarbonate, BUN, calcium, chloride, cholesterol, and the like typically remain within normal levels and do not undergo unacceptable adverse changes. Upon administration of the chelator conjugate, urinalysis typically reveals no significant change in creatine clearance or urine output. Hematological measurements such as white blood cell and red blood cell counts, hemoglobin, hematocrit, platelet count, percentages of lymphocytes, monocytes, granulocytes, eosinophils, basophils, and morphology typically remain within the normal ranges and exhibit no unacceptable adverse changes such as decrease of red blood cell counts or increases in white blood cell counts. Vital signs

such as systolic and diastolic blood pressures, oral temperature, heart rate, and respiratory rate are not unacceptably adversely affected by treatment of iron overload disorders by administration of a chelator conjugate of a chelator and a water soluble carrier.

In a preferred embodiment of the invention, an iron overload disorder is treated by administering a chelator conjugate deferoxamine bound to a water-soluble carrier, preferably hydroxyethyl starch. Studies of the toxicity and other effects of a deferoxamine-hydroxyethyl starch (DFO-HES) chelator conjugate in non-human animals are reported in the examples that follow. In addition, a DFO-HES chelator conjugate has been administered to humans, shown to have acceptable acute toxicology, and shown increase urinary iron excretion in patients with two iron overload disorders. These results are also presented in the examples below.

The invention will be further described by reference to the following detailed examples, which are illustrative of but not limiting to the present invention.

EXAMPLES

Example 1 -- The Effect of Deferoxamine Treatment on Urinary Iron Excretions

Eight patients, three with Diamond-Blackfan anemia and five with thalassemia were administered deferoxamine in a standard treatment protocol and levels of urinary iron excretion were monitored. The

standard treatment protocol for deferoxamine is a daily 8-12 hour subcutaneous infusion of deferoxamine to a total dose of 50 mg/kg. Complete urine collections were made during a 24 hour period and urinary iron concentration was determined by atomic absorption spectroscopy. The results for each patient are recorded in Table 1.

Table 1
Urinary Iron Excretion

Patient Number	Daily Fe excretion with a single dose of DFO at 50 mg/kg (Historical data)	Daily Fe excretion with a single dose of DFO at 50 mg/kg (During trial)
1	12.2 mg	5.9 mg
2	13.4 mg	7.8 mg
3	16.3 mg	+
4	43.4 mg	+
5	36.0 mg	12.5 mg
6	16.9 mg	29.3 mg
7	13.7 mg	27.6 mg
8	14.9 mg	23.2 mg

Patients 1-4 and 7 suffer from β-thalassemia. Patients 5, 6 and 8 suffer from Diamond-Blackfan anemia. Data for patients 3 and 4 during the trial of HES-DFO administration were unavailable because the patients refused further deferoxamine therapy. Each value is an average of several measurements.

Regular, daily 8-12 hour, continuous subcutaneous infusion with deferoxamine lead to excretion from these β-thalassemia patients of an average of 17.5 mg of iron per day and from these Diamond-Blackfan Anemia patients of 22.1 mg of iron per day.

Example 2 -- Toxicity of HES-DFO in Mice

Study Drug

The HES-DFO (hydroxyethyl starch deferoxamine conjugate) used for this study was produced by chemically attaching the iron chelator deferoxamine to hydroxyethyl starch. The resulting chelator conjugate is polydisperse, with a weight average molecular weight between 50,000 and 200,000. It was supplied as a pale yellow aqueous solution at a total drug polymer concentration of 100 g/L. The total chelator concentration is approximately 26 mM. The drug solution also contains approximately 0.9% sodium chloride, sufficient to bring the osmolarity of the drug to 290-330 mOsm. The drug was typically provided in vials containing approximately 240 mL. The study drug was stored refrigerated (2-8°C). It can be kept at ambient temperature during administration for a period of time not to exceed 30 hours.

Administration to Mice

Acute toxicity of intravenous administration of HES-DFO has been investigated in mice. Animals were given two intravenous injections of HES-DFO approximately 6 hours apart. Doses given were 45, 65, and 85 mL/kg at each dosing, so that the maximum does given in the six hour pried was 170 mL/kg. For reference, the maximum dose given to human patients in subsequent clinical trials was 9 mL/kg. There were 10

mice per each dose group, plus a single group of 10 mice that received saline at 170 mL/kg (in two doses).

Results showed two deaths out of ten mice at the time of dosing in the lowest dose group, and one each in the two higher dose groups. Hence, the incidence of deaths was apparently not dose-related. Macroscopic findings postmortem, which consisted of an external examination and a detailed examination of the major body cavities, organs, and viscera, were considered incidental and of no toxicological significance.

Based on the results of this study, administration of 10 g% HES-DFO chelator conjugate is well tolerated up to total daily dose volumes of 170 mL/kg when given in equally divided doses to mice. It appears that HES-DFO has low inherent acute toxicity based on the large volume of material administered. Volume-related effects were anticipated because vascular load was increased 1-3 times; however, effects that could be related to volume were not observed. Clinical signs of prostration and convulsions were noted in one or more high dose animals which may be related to altered toxicity and changes in ionic fluxes.

Example 3

Hemodynamics in Dogs

Three publications report studies of hemodynamic effects of HES-DFO in dogs. These studies conclude that HES-DFO does not adversely affect

hemodynamic measures in dogs. Briefly, these studies administered intravenous or intraatrial HES-DFO at doses from 3 to 6 mL/kg. No changes in hemodynamics (such as blood pressure, cardiac output, or heart rate) were observed. Equivalent doses of DFO alone caused significant hypotension.

These studies are described in more detail in the following articles: J.R. Forder, T.B. McClanahan, K.P. Gallagher, B.E. Heldund, P.E. Hallaway, M. Shlafer, "Hemodynamic Effects of Intraatrial Administration of Deferoxamine or Deferoxamine-Pentafraction Conjugate to Conscious Dogs", J. Cardiovascular Pharmacology 16, 742-749 (1990); P.E. Hallaway, J.W. Eaton, S.S. Panter, B.E. Hedlund, "Modulation of Deferoxamine Toxicity and Clearance by Covalent Attachment to Biocompatible Polymers", Proc. Natl. Acad. Sci. USA 86, 10108-10112 (1989); M. Maruyama, G.M. Pieper, B. Kalyanaraman, P.E. Hallaway, B.E. Hedlund, G.J. Gross, "Effects of Hydroxyethyl Starch Conjugated Deferoxamine on Myocardial Functional Recovery Following Coronary Occlusion and Reperfusion in Dogs", J. Cardiovascular Pharmacology 17, 166-175 (1991). These three references are incorporated herein by reference.

Example 4

Treatment of Normal volunteers with HES-DFO

Summary and Conclusions

This study determined the safety and pharmacokinetics of intravenous infusion of HES-DFO.

It was a rising single dose, double blinded parallel study involving sixteen healthy male patients under fasting conditions. Four groups of four patients each were dosed, with three patients in each group receiving HES-DFO and the fourth randomly chosen patient receiving 0.9% sterile sodium chloride injection as placebo. The dose levels tested were 0.3, 0.9, 3.0 and 9.0 mL/kg. The HES-DFO or placebo was administered by intravenous infusion over a four hour period, with the infusion rate adjusted so that 1/3 of the dose was given in the first 15 minutes and the remaining two thirds of the dose given in the subsequent 3 hours and 45 minutes. Dosing started with the lowest dose group and proceeded to the next dose level only after the preceding dose was judged to be well tolerated.

The study monitored patient vital signs, clinical blood chemistry, hematology, urinalysis, ECG, clotting parameters, and renal clearance during the drug infusion and for 7 days after dosing. In addition, plasma HES-DFO concentrations were measured at various times up to four weeks after dosing, and the amount of HES-DFO and iron passed in the urine within 48 hours after dosing was determined. All sixteen patients completed the study. No serious adverse events or any events required terminating any patients from the study. The clinical blood chemistries, clotting parameters, urinalysis, hematology, renal clearance, ECG readings, and vital signs were unaffected by HES-DFO administration over

the course of the study. Normal variations in clinical blood chemistry parameters were noted across all dosage groups and time periods. No systematic changes in lab parameters were noted that could be related to drug dosing.

Pharmacokinetic analysis showed that the plasma drug levels peaked at the end of the four hour infusion, and peak plasma drug levels reached 3 mM chelator concentration in the highest dose group. The analysis revealed non-exponential decay in plasma drug levels, with 15% of the peak drug level present at 7 days and declining to approximately 4% present by 4 weeks post dosing. From 35 to 55% of the total amount of drug dosed was excreted in the urine in the first 48 hours after dosing. Urinary iron excretion increased in patients receiving HES-DFO.

HES-DFO was well tolerated at all doses. There were no observations that would cause concern over the drug's use in clinical trials.

Study Drug

Study drug was prepared as described in Example 2.

Blinding

This study was double blinded. The blinding was accomplished using a pharmacist not conducting the clinical measurements to prepare and administer the test material or placebo. The use of the pharmacist was necessary because the test solution had a pale

yellow color which might have been distinguishable from the placebo. The bottles containing either the sodium chloride injection or the HES-DFO and the infusion lines were wrapped in foil by the pharmacist out of sight of all other staff to prevent the possibility of observation of the solution identity by either the study patients or the personnel monitoring the dosing. The assignment to the sodium chloride or study drug was done using a random number table by an employee of the sponsor who was not directly involved in the study, and the dosing key was known at the clinical site only by the pharmacist who set out the IV solutions for each patient. The blinding was not broken until all clinical observations and blood samplings were completed at the end of the study.

Patient Selection

Patients were volunteer university students and non-institutionalized members of the community at large. All volunteers selected for this study were males of 18 to 40 years of age, inclusive. The weight range of the volunteers was not more than ±10% for height and body frame as per Desirable Weights of Men -- 1983 Metropolitan Height and Weight Table.

Patients completed all screening procedures within two weeks of entry into the study. The screening visit(s) included collecting the following information: medical history, medication history, physical examination, 12-lead ECG, blood pressure, heart rate, respiratory rate and aural temperature.

Patients were considered normal by medical history, medication history, physical examination, 12-lead ECG, blood pressure, heart rate, respiratory rate, and body temperature.

Patient Treatment and Study Schedule

Patients selected to enter a dosing group reported to the clinical testing site the evening before dosing was scheduled. The informed consent document was reviewed and signed again by the patient and a witness. Patients remained confined to the clinical unit for 48 hours after beginning dosing. Meals and fluid intake were controlled during the confinement period.

Following the evening meal on day -1, patients were fasted for at least 10 hours prior to dosing and for five hours following commencement of dosing. On the morning of dosing (day 1), vital signs and baseline blood and urine samples were taken within on hour prior to starting dosing. Additional blood samples for hematology and clinical chemistries were than taken at 1, 4, 24, and 48 hours after dosing and on day 8. Blood samples for coagulation parameters were taken at 24 and 48 hours after dosing, and blood samples for drug content were taken 12 times in the first 48 hours, and on days 5 and 8. Additional blood draws for drug content analysis on days 15 and 29 were subsequently added to the protocol after the trial had started in order to follow the decay in plasma HES-DFO content to lower levels. The additional blood draws

for plasma drug levels on day 15 were not collected for group 1, however. This was due to the fact that the protocol amendment for the additional samples was not made until after day 15 for group 1. The day 29 samples were collected for group 1.

Twelve-lead electrocardiograms were taken within one hour prior to dosing, and at 30 minutes, and 1, 2, 4, 24, and 48 hours after beginning dosing.

Urine sampling for drug content analysis and for urinalysis and creatinine, sodium, and potassium clearance was done within one hour prior to dosing and after dosing over 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 12, 12 to 24, and 24 to 48 hours.

Patients were monitored throughout the confinement portion of the study. Vital signs such as blood pressure, heart rate, and temperature were monitored every 5 minutes for the first 15 minutes of infusion, then every 15 minutes during the infusion, every thirty minutes for two hours after the infusion had been completed and then every 2 hours thereafter for an additional 6 hours and again at 12, 24, 36, and 48 hours post start of dosing (through day 3 of the study).

Patients were discharged from the clinical study unit on day 3 (48 hours after starting dosing) following an exit physical exam. They returned to the study unit on days 5, 8, 15, and 29 for the additional blood draws required for clinical chemistry, hematology and plasma drug concentration determinations.

Vital Signs

Systolic and diastolic blood pressures, aural temperatures, heart rate, and respiratory rates ere measured during the course of the trial. No significant deviations in these parameters were discernible.

Particular attention was paid to the possibility of hypotensive episodes, since this is a well known side effect of deferoxamine mesylate (Desferal®). There was no evidence of drug-induced blood pressure changes.

Clinical Chemistry Results

Only normal variations in clinical chemistry parameters were noted across all dosage groups and time periods. With the exceptions of plasma iron and phosphorous, whose assays were shown to be not valid in the presence of HES-DFO, no systematic changes in any clinical chemistry parameters were noted that could be related to drug dosing. That is, with the exception of iron and phosphorous, no clinical chemistry measurements were noted that deviated systematically with higher drug doses, with the timing of plasma drug levels, or that were outside of normal limits in any patient receiving HES-DFO.

Hematology Results

Hematology measures, which included white blood cell and red blood cell counts, hemoglobin,

hematocrit, platelet count, percentages of lymphocytes, monocytes, granulocytes, eosinophils, basophils, and morphology appeared to be unaffected by drug dosing. Occasional out of normal range results were seen across all dosage groups (including the controls) and times.

Urinalysis Results

The urinalysis measures, which consisted of a standard panel of 17 measures performed at 9 times throughout the trial, were all within normal limits with the sole exception of five individual measurements in three individuals in group 2. One of these received control (sodium chloride) solution, and the other two HES-DFO. These results were considered not to be of clinical significance.

Coagulation Parameters

Partial thromboplastin time (PTT) and prothrombin time (PT) were measured immediately prior to dosing and at 24 and 48 hours after dosing. Both the PTT and PT measures appeared to be unaffected by HES-DFO dosing. Of the 48 measurements performed, only two PTT measurements in two individuals were slightly outside of normal limits at the 24 and 48 hour time points. One of these was the control patient in group 1, who had PTT values of 26.4 and 24.8 seconds, and the other was a patient receiving HES-DFO in group 3 who had values of 26.4 and 25.6 seconds. These values are slightly below the

laboratory normal reference range of 26.5 to 36.5 seconds. A single patient receiving HES-DFO in group 4 had a single out of normal range value for prothrombin time of 13.2 seconds, slightly outside the normal reference range of 11 to 13 seconds. There is no apparent affect on PT and PTT from the HES-DFO infusion.

Renal Clearance

Creatinine clearance was determined for the 0 to 24 hours and 24 to 48 hours periods, and sodium and potassium clearances were determined for the 0 to 24 hour period. No values were seen that fell below normal limits at any time during the study. No evidence of kidney impairment was found. Urine output was unaffected.

Electrocardiogram

Each patient received six 12-lead electrocardiograms during the period from 30 minutes to 48 hours after starting dosing. Of these 96 ECG readings, none were judged to contain clinically significant abnormal events.

Example 5 -- Treatment of Patient with Thalassemia or Diamond-Blackfan Anemia with HES-DFO

Patient Selection

A phase I/II study of the safety, pharmacokinetics, and effect on plasma and urinary iron concentrations was conducted on 8 patients with

iron overload disorders. Three of the patients suffered from Diamond-Blackfan anemia. Five of the patients suffered from β-thalassemia.

Each patient was greater than 6 years of age and had a positive diagnosis of homozygous β-thalassemia or Diamond-Blackfan anemia, and required regular transfusions. Each patient had either a liver iron content greater than 6 mg iron per gram dry weight of liver, as measured on liver biopsy or by use of a superconducting quantum interference device (SQUID), or serum ferritin concentrations of at least 1,500 ng/mL prior to entering the trial. Patients with certain conditions were excluded. Excluded patients include those with a glomerular filtration rate outside the normal range and patients with a known history of allergic responses to hydroxyethyl starch, deferoxamine, HES-DFO, or related drugs.

Study Drug

Study drug was prepared as described in Example 2.

Patient Treatment and Study Schedule

The patients will have ceased other chelation therapy for 48 hours prior to receiving the HES-DFO infusion. No other chelators were taken during the 7 days following HES-DFO administration. In addition, there was an interval of 2 to 9 days since the last blood transfusion before the patient

was given the HES-DFO infusion. No blood transfusions were given for 7 days after administering HES-DFO.

Patients entered the clinical unit the morning of the start of drug infusion (Day 1) and remained in the clinical unit until departure on the afternoon of study day 5. A peripheral IV line for drug infusion was inserted on the morning of day 1, and the HES-DFO administration was accomplished by a single intravenous infusion over a period of sixty minutes. After completion of the dosing, patients were ambulatory. Vital signs monitored and laboratory and clinical assessments were made as described above in Example 4. Special attention was directed toward signs of hypo- or hypertension, tachycardia, uticharia, flushing, gastrointestinal syndrome, ocular and auditory disturbances, and decreased renal output.

Each patient received a single dose of the study drug HES-DFO by intravenous infusion over sixty minutes. The doses investigated were 3 and 5 mL/kg. Four patients were scheduled to receive the lowest dose (3 mL/kg), and four received the highest dose (5 mL/kg). Patients remained in the clinical unit for 5 days after dosing, and returned on or around days 8, 15, 22 and 29 for drawing of blood for drug level determinations, hematology, and clinical chemistry. Twenty-four hour urinary collections were performed daily during the five-day stay in the clinical investigation unit, and during days 6 and 7 at home after discharge. The collected urine was analyzed for HES-DFO and iron. Plasma and urine drug

and iron levels, vital signs, hematology, blood chemistry, clotting parameters, serum ferritin concentration, and urinalysis were monitored over the course of the trial.

All doses were completed as scheduled with the exception of patient #2, who was scheduled to receive 3 mL/kg. This patient experienced an adverse reaction to the infusion, and infusion was stopped after having received approximately 10% of the scheduled dose. This patient, who has a very high level of anxiety, has been noted in the past to have the same symptoms during non-invasive investigations, including magnetic resonance imaging and x-ray studies. It is the opinion of the physicians that at least in part these symptoms were due to the anxiety of the patient.

Plasma and Urine Drug and Iron Levels

Plasma HES-DFO levels, expressed as the concentration of high molecular weight chelator in plasma, are shown in graphical form in Figures 1 and 2. Peak plasma HES-DFO levels were between 0.8 and 1.48 mM in the patients who received 3 mL/kg, and between 1.6 and 2.4 mM in the patients who received 5 mL/kg.

Urine HES-DFO and urine iron excretion are shown graphically in Figures 3-6, and summarized in Table 2. Urinary iron excretion ranged from 22.9 to 49.8 mg in 7 days at the low dose (3 mL/kg). At the high dose of 5 mL/kg, the Diamond-Blackfan

patients excreted from 21.2 to 29.8 mg of iron in one week, and the single thalassemia patient treated with this dose excreted 96.3 mg iron in 7 days. Table 2 compares these results with the urinary iron excretion while using DFO, from both historical data and from 24 hour urine collections taken after the first week on the trial at which time DFO administration was permitted.

Table 2
Urinary Iron Excretion

Patient number and dose of HES-DFO chelator conjugate administered	Weekly Fe excretion with a single dose of HES-DFO	Fe excretion with a single dose of DFO at 50 mg/kg (Historical data)	Fe excretion with a single dose of DFO at 50 mg/kg (During trial)
1, 3 mL/kg	22.9 mg	12.2 mg	5.9 mg
2, ~0.3 mL/kg	7.7 mg	13.4 mg	7.8 mg
3, 3 mL/kg	28.7 mg	16.3 mg	+
4, 3 mL/kg	49.8 mg	43.4 mg	+
5, 5 mL/kg	21.5 mg	36.0 mg	12.5 mg
6, 5 mL/kg	21.2 mg	16.9 mg	29.3 mg
7, 5 mL/kg	96.3 mg	13.7 mg	27.6 mg
8, 5 mL/kg	29.8 mg	14.9 mg	23.2 mg

Patients 1-4 and 7 suffer from β-thalassemia. Patients 5, 6 and 8 suffer from Diamond-Blackfan anemia. Data for patients 3 and 4 during the trial of HES-DFO administration were unavailable because the patients refused further deferoxamine therapy. Each value is an average of several measurements.

From these results, it appears that a single dose of HES-DFO at 3 mL/kg stimulates the same amount of urinary iron excretion as 1.1 to 3.9 days of DFO in thalassemia patients. At 5 mL/kg in the Diamond-

Blackfan patients, urinary iron excretion was between 59% and 172% of one day's excretion using DFO. The single thalassemia patient dosed at 5 mL/kg excreted 3.5 to 7.0 times as much iron in a week as with DFO in 24 hours.

Pharmacokinetics

The AUC and AUMC were calculated form the plasma HES-DFO concentration vs. time data (graphed in Figures 1 and 2). Average clearance over the four week period of the trial was calculated as the Dose ÷ AUC. The mean residence time (MRT) of the drug was calculated as AUMC/AUC. The first $T_{1/2}$ is the time estimated (by interpolation of the curves in Figures 1 and 2) for the plasma drug levels to decay from maximum to half maximum. The second $T_{1/2}$ is the time required for the plasma drug levels to decay from half maximum to quarter maximum. Finally, the 7 day efficacy is the total iron excreted in the urine over 7 days divided by the maximum chelating capacity of the infused dose (mole/mole). Table 3 gives the results of these calculations for the 7 patients who received their complete scheduled HES-DFO doses.

Due to the polydispersity of HES-DFO, several of these quantities should be interpreted as estimates, particularly those involving integration over the entire plasma lifetime of the drug. This includes the AUC, AUMC, Clearance, MRT, and Vd. Appropriate indicators of the drug's pharmacokinetics include the plasma clearance rates of HES-DFO calculated as a

function of time after infusion. Renal clearance plots for both HES-DFO and for plasma iron were calculated for each urine collection period using the mean plasma concentration of HES-DFO or iron for that time period. Both iron and HES-DFO clearances decline rapidly after infusion of the drug. A possible reason for this is that the smaller, more readily excreted, polymer is cleared first and only molecules that break

Table 3

Pharmacokinetic Calculations for HES-DFO

Patient #	Dose (mL/kg)	AUC (mMoles-hr/L)	AUMC (mMoles-hr^2/L)	HES-DFO Clearance (mL/min)	MRT (hours)	Vd (L)	First $T_{1/2}$ (hours)	2nd $T_{1/2}$ (hours)	7 day efficacy
1 (Thal)	3 mL/kg	63.5	11,950	1.043	188	11.7	13.5	42.5	10.3%
3 (Thal)	3 mL/kg	88.5	17,365	0.752	196	8.8	7.2	25.5	12.9%
4 (Thal)	3 mL/kg	93.2	22,550	0.965	242	14.0	17.9	67.3	16.9%
5 (D-B)	5 mL/kg	142.8	29,628	0.657	207	8.2	52.2	59.9	6.8%
6 (D-B)	5 mL/kg	141.7	22,923	0.691	161	6.7	4.2	62.7	6.5%
7 (Thal)	5 mL/kg	119.0	21,254	1.103	179	11.8	19.9	46.3	21.9%
8 (D-B)	5 mL/kg	170.3	26,196	0.771	154	7.1	23.1	71.0	6.8%

SUBSTITUTE SHEET (RULE 26)

down or are excreted more slowly continue to circulate, and hence are cleared more slowly. Renal clearance of HES-DFO is relatively consistent among the patients.

Non Transferrin-Bound Iron (NTBI)

Plasma samples were analyzed for NTBI by Dr. John Porter, Department of Haematology, University College, Lond, UK. These measurements report concentrations of low molecular weight, non-deferoxamine bound iron, which has been suggested to be a source of long term damage in iron overload patients. Thalassemia patients who are not being chelated show elevated levels of NTBI. DFO infusion brings these levels down to zero within a few hours. On cessation of DFO infusion, however, the elevated NTBI levels return within an hour. See, Singh, Hider, and Porter, Anal. Chem. 180, 320-323 (1990); Porter, Abeysinghe, Marshall, Hider, and Singh, Blood 88, July 15, 1996. The measured NTBI levels of the patients in the trial were given in Table 4. Infusion of HES-DFO brings these levels down to zero. They remain at zero for 1 to 4 days, at which time they rebound, occasionally to levels higher than starting values. The point at which they begin to rise correlates well with the point at which the circulating chelator concentration falls below the total plasma iron concentrations (Figures 1 and 2).

TABLE 4

Non Transferrin-Bound Iron Levels in Each Patient

Time (hr)	#1 NTBI (μM)	Time (hr)	#2 NTBI (μM)	Time (hr)	#3 NTBI (μM0	Time (hr)	#4 NTBI (μM)	Time (hr)	#5 NTBI (μM)	Time (hr)	#6 NTBI (μM)	Time (hr)	#7 NTBI (μM)	Time (hr)	#8 NTBI (μM)
0	NS	0	4.69	0	2.17	0	3.01	0	4.92	0	0.107	0	4.27	0	3.75
1	NS	1	0.07	1	0.49	1	-0.35	1	-0.12	1	-0.35	1	-0.35	1	-0.12
4	NS	4	0.28	4	-0.35	4	-0.35	4	-0.12	4	-0.35	4	-0.35	4	-0.12
12	0.07	12	1.33	12	0.07	12	0.07	12	-0.12	12	-0.35	12	-0.35	12	-0.12
24	3.85	24	5.53	24	0.07	24	0.07	24	-0.12	24	-0.35	24	0.07	24	-0.12
48	0.49	48	5.95	48	0.07	48	0.07	48	NS	48	0.49	48	25.7	48	0.65
96	3.85	96	5.11	93	1.33	96	4.27	96	0.27	96	0.49	96	18.13	96	2.59
170	3.85	169	3.43	194	7.63	218	9.73	193	6.08	168	18.13	168	13.51	168	20.81
339	1.33	338	1.33	361	7.2	387	6.79	366	6.47	336	4.69	339	10.15	336	5.3
507	0.07	505	0.49	572	5.53	534	5.53	535	8.02	504	3.43	557	11.41	528	5.69
675	NS	673	2.59	743	4.69	672	5.11	703	5.69	696	8.89	676	3.43	NS	NS

Safety Data

There were no changes in clinical chemistry results that correlated with HES-DFO administration. No changes were noted that could be attributed to HES-DFO dosing. There appeared to be no significant changes in vital signs throughout the trial, with the exception of patient #2.

Conclusions

Because of the short-term nature of this study, the measurement used to estimate efficacy of HES-DFO was urinary iron excretion induced for the seven days following administration of a single intravenous dose of HES-DFO infused over 60 minutes. The doses used were 3 and 5 mL/kg. The results of urinary iron excretion induced by HES-DFO are summarized in Section II.

Efficacy of a single dose of HES-DFO in 4 evaluable patients with thalassemia major was evaluated. HES-DFO at either 3 or 5 mL/kg appears to induce, over 7 days, in patients with thalassemia major, iron excretion equal to the excreted by patient with thalassemia major during 1.1 to 7.0 days of 8-12 hours daily of administration of subcutaneous deferoxamine (according to historical iron excretion data), and during 0.7 to 3.8 days of 8-12 hours administration of subcutaneous deferoxamine (according to recent iron excretion data). The higher excretion

is observed, as expected, at the higher doses of HES-DFO.

Efficacy of a single dose of HES-DFO in 3 evaluable patients with Diamond-Blackfan anemia was evaluated. HES-DFO at 5 mL/kg appears to induce, over 7 days in patients with Diamond-Blackfan anemia, iron excretion equal to that excreted by the same patients during 0.6 to 2.0 days of 8-12 hours daily of administration of subcutaneous deferoxamine (according to historical iron excretion data), and during 0.7 to 1.7 days of 8-12 hours daily of administration of subcutaneous deferoxamine (according to recent iron excretion data).

Studies of NTBI showed that infusion of HES-DFO reduced plasma concentrations of NTBI to zero or very low levels, for periods varying between 12 and 96 hours. These findings and the return to baseline values after HES-DFO concentrations declined below those of plasma iron are consistent with the return of NTBI after discontinuation of DFO infusions

The efficacy and lack of toxicity of HES-DFO in this short-term study suggest that this compound plays a useful role in therapy of patients with iron overload disorders and should be considered for further studies. In the clinical setting, it would be useful to have a compound with efficacy such as that observed in this study. This would represent a definite improvement in the therapy and quality of life for patients with thalassemia major, Diamond-Blackfan anemia and other iron overload disorders.

The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention. All publications and patent applications in this specification are indicative of the level of ordinary skill in the art to which this invention pertains.

CLAIMS

1. Use of a covalently bonded conjugate of a chelator and a water soluble carrier for use in the manufacture of a medicament for treating an iron overload disorder in a patient, the method comprising administering the conjugate to the patient.

2. The use of claim 1, wherein the iron overload disorder is thalassemia, sickle cell disease, Diamond-Blackfan anemia, hereditary (idiopathic) hemochromatosis, hereditary transferrin deficiency, thalassemia syndromes, hereditary hypochromic anemia, African hemosiderosis, Kaschin-Beck disease, transfusional hemosiderosis, alcoholic cirrhosis with hemosiderosis, porphyria cutanea tarda, acquired hemolytic anemia, ineffective erythropoiesis, or pyridoxine-responsive anemia.

3. The use of either preceding claim, wherein the patient is a child, adolescent or adult human.

4. The use of any of claims 1 to 3, wherein the administration is parenteral.

5. The use of any of claims 1 to 3, wherein the administration is intravenous.

6. The use of any preceding claim, wherein the water soluble carrier is starch, a starch derivative, dextran, or hyaluronic acid.

7. The use of any preceding claim, wherein the chelator is deferoxamine.

8. The use of claim 7, wherein the water soluble carrier is hydroxyethyl starch.

9. The use of claim 8, wherein the hydroxyethyl starch has an average molecular weight of about 50 kD to about 200 kD.

10. The use of any of claims 7 to 9, wherein the chelator conjugate is administered as a solution with chelator conjugate concentration at about 100 g/L to about 200 g/L.

11. The use of any of claims 7 to 10, wherein the chelator conjugate is administered as a solution with a total deferoxamine concentration of about 26 mM to about 30 mM.

12. The use of any of claims 7 to 11, wherein the chelator conjugate is administered at about 3 mL/kg t about 9 mL/kg.

13. The use of any of claims 7 to 12, wherein an amount of conjugate administered is sufficient to achieve plasma levels of chelator of about 0.5 mM to about 3 mM.

14. The use of any of claims 7 to 13, wherein the administration is by intravenous infusion for a period of less than about 3 hours.

15. The use of any preceding claim, wherein the chelator conjugate is administered in a pharmaceutically acceptable vehicle.

16. The use of claim 15, wherein the pharmaceutically acceptable vehicle is water including about 0.9% sodium chloride.

17. The use of any preceding claim, wherein the chelator conjugate is

administered as a solution with chelator conjugate concentration of about 5 g/L to about 250 g/L.

18. The use of any preceding claim, wherein the chelator conjugate is administered as a solution with a total chelator concentration of about 5 mM to about 100 mM.

19. The use of any preceding claim, wherein an amount of conjugate administered is sufficient to achieve plasma levels of chelator of about 0.1 mM to about 15 mM.

20. The use of any preceding claim, wherein the chelator conjugate is administered at about 1 mL/kg to about 30 mL/kg.

21. Use according to any preceding claim, wherein the treatment comprises administering an effective amount of the conjugate to the patient and monitoring iron status of the patient.

22. The use of any preceding claim, wherein the treatment further comprises monitoring plasma iron concentration.

23. The use of any preceding claim, wherein the method further comprises monitoring urinary iron concentration.

24. The use of any preceding claim, wherein the treatment comprises making laboratory and clinical assessments of the patient.

25. The use of any preceding claim, wherein the treatment further

comprises monitoring total body iron burden.

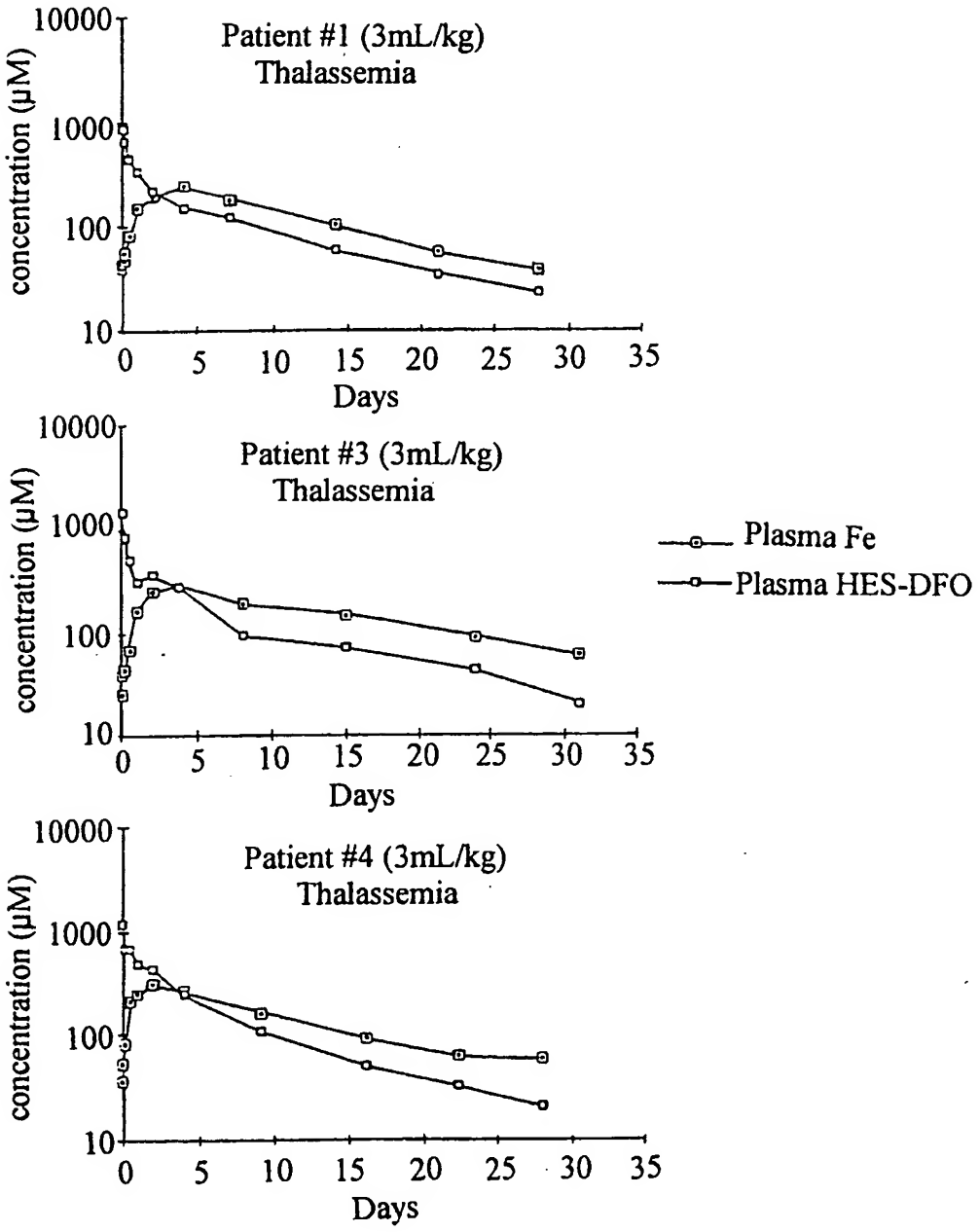
26. The use of any preceding claim, wherein the administration results in excretion of about 20 mg to about 100 mg urinary iron per 7 days.

27. A covalently bonded conjugate of a chelator and a water soluble carrier, the conjugate being as specified in any preceding claim.

28. A covalently bonded conjugate of a chelator and a water soluble carrier, the conjugate being for use in a method of treatment of the human or animal body by therapy.

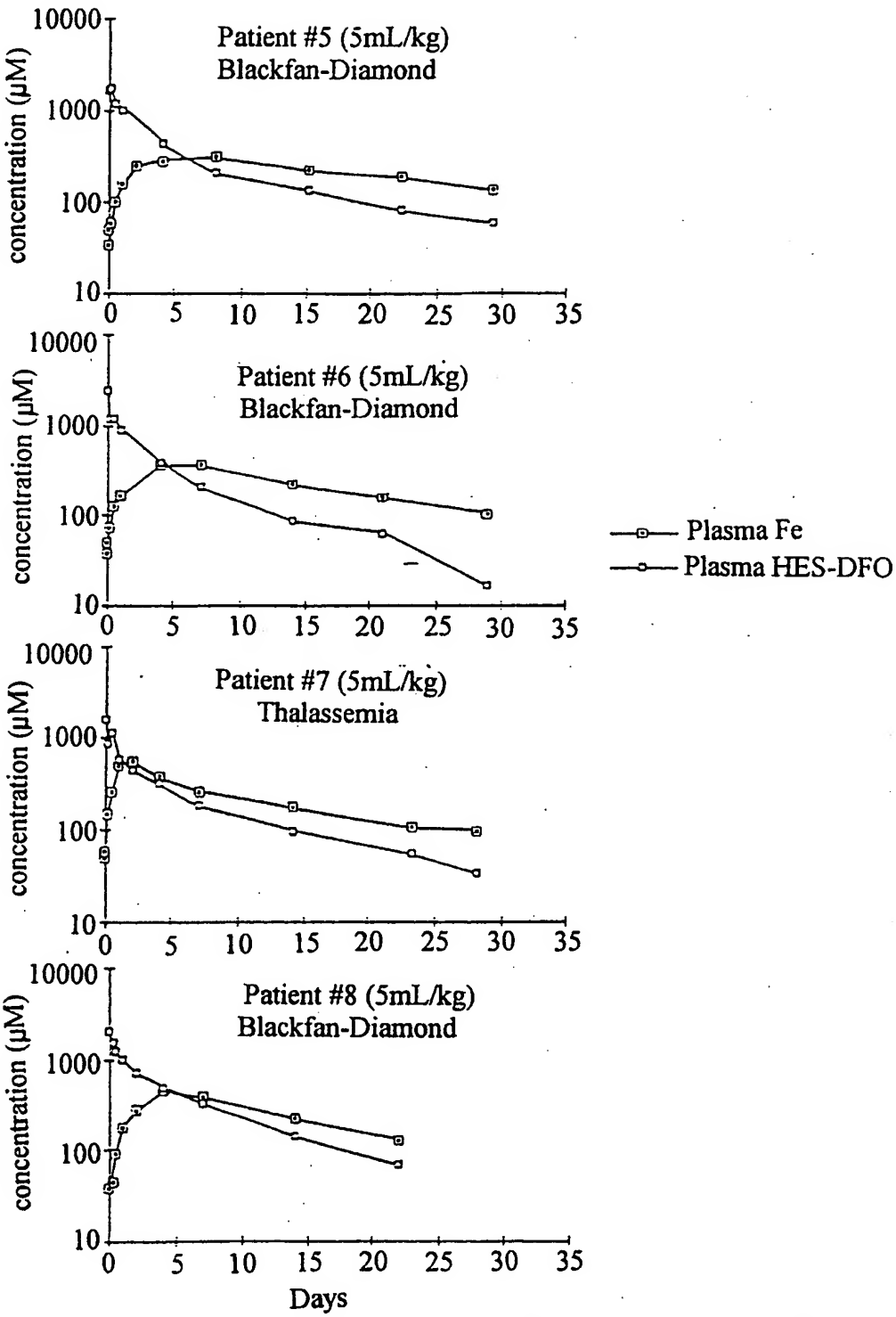
29. A method of treating an iron overload disorder in a patient, the method comprising administering to the patient a covalently bonded conjugate as specified in any of claims 1 and 6 to 9.

30. A method according to claim 29, wherein the method comprises a feature as specified in any of claims 2 to 5 and 10 to 26.



Plasma iron and HES-DFO concentrations following dosing in the three patients who received 3 mL/kg.

FIG .1



Plasma iron and HES-DFO concentrations following dosing in the four patients who received 5 mL/kg.

FIG. 2

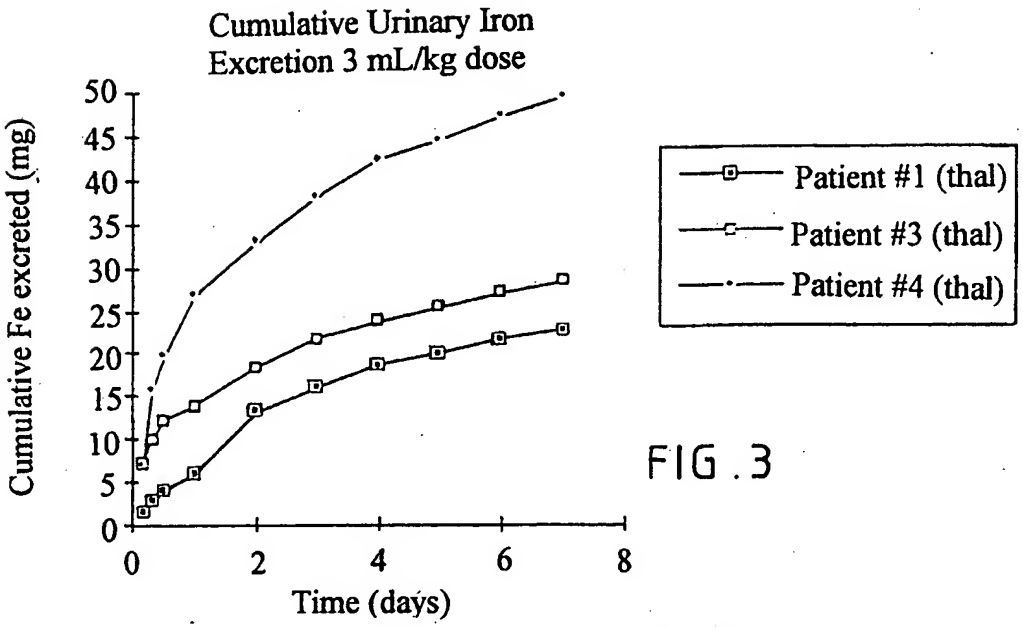


FIG. 3

Cumulative urinary iron excretion at 3 mL/kg dose.

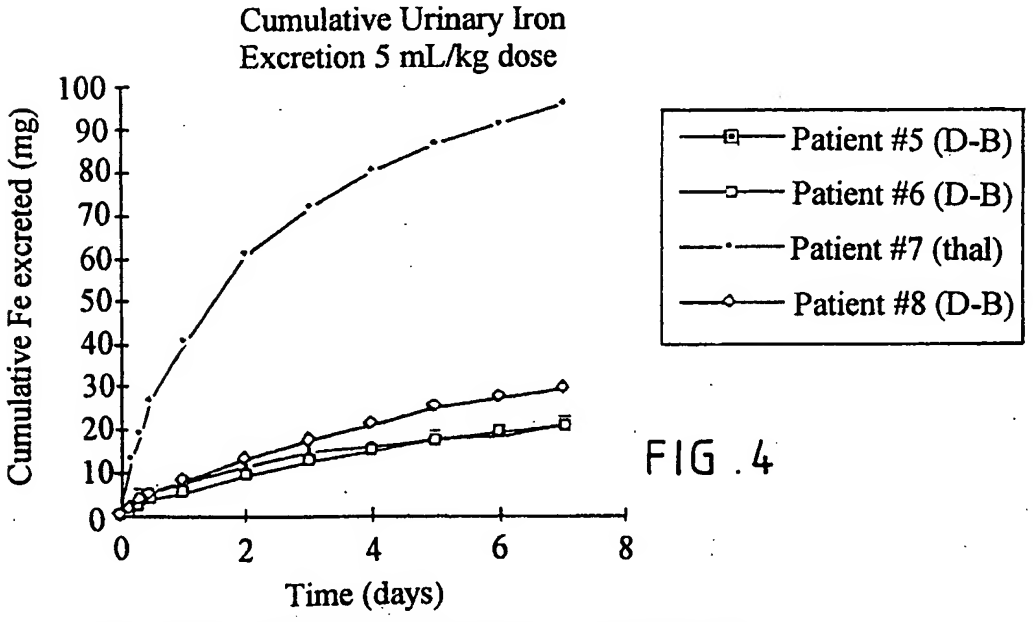


FIG. 4

Cumulative urinary iron excretion at 5 mL/kg dose.

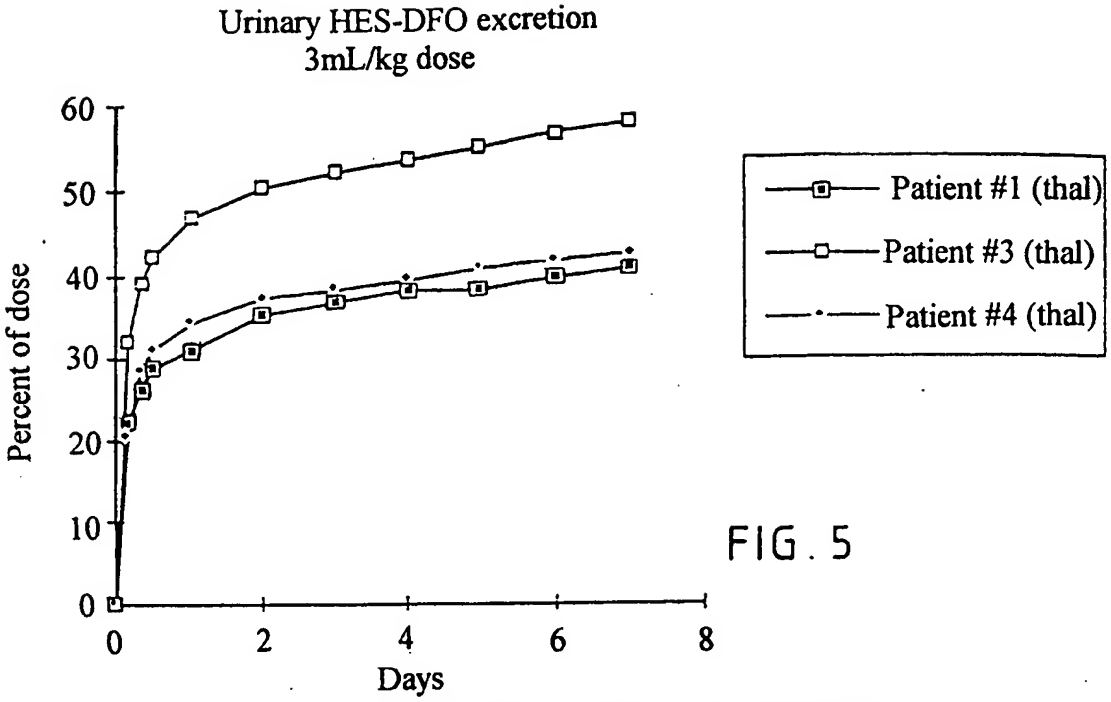


FIG. 5

Cumulative urinary HES-DFO excretion at 3mL/kg dose.

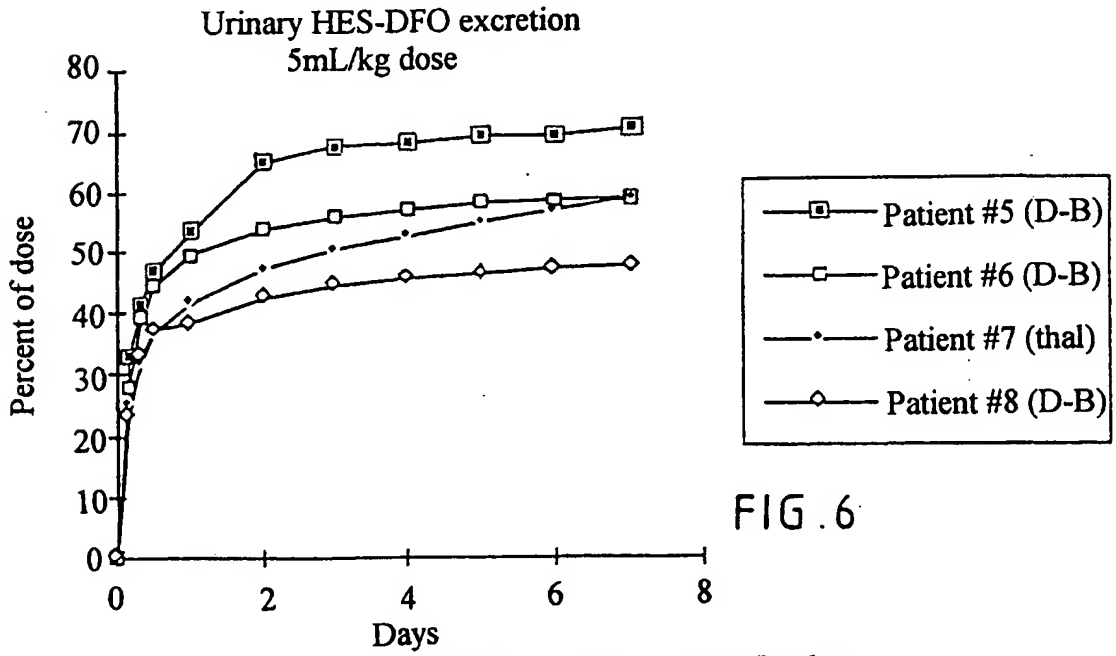


FIG. 6

Cumulative urinary HES-DFO excretion at 5mL/kg dose.